INTEGRATIVE PATTERN OF VASOMOTOR EFFICIENCY IN SHR DURING ONTOGENESIS.

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Short title: Vasomotor efficiency in SHR in ontogenesis

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Summary

Wealthy data from hundreds studies concerning the cardiovascular system in SHR are often controversial. The background of diversity has different roots, among them (i) different vascular segments or areas were studied, (ii) the experimental objects were of different ontogenetic period. The study aimed to follow the BP as an integrated response of vascular system. This access was justified since stabilized cardiac output in SHR was proved till 1 year of age. The groups of SHR, males, 3-5-9-17-52 weeks of age and age matched Wistar rats were used. Significant steady state BP difference between SHR and Wistar rats was found in 9 weeks of age and continued till 52 weeks, representing 189.6±11.9 mmHg in SHR and 117.3±6.9 mmHg ($P<0.01$) in Wistar rats. The significant difference in BP increase to two doses of noradrenaline (0.1μg and 1μg) between SHR and control rats was found in the same age. In 52 weeks the BP increment to two doses was in SHR 19.7±2.0 mmHg and 60.5±3.9 mmHg and in Wistar rats 7.4±1.9 mmHg and 40.5±3.2 mmHg ($P<0.01$). The hypotensive response to acetylcholine (0.1μg, 1μg and 10μg) increased dose dependently in SHR, it was amplified in 17 weeks of age only and persisted to 52 weeks. In SHR 52 weeks old the hypotensive response to 3 doses amounts 69.9±10.2 mmHg, 87.5±11.8 mmHg and 103.4±10.6 mmHg, in Wistar rats 37.4±4.2 mmHg ($P<0.01$), 62.3±3.5 mmHg ($P<0.01$) and 73.5±2.8 mmHg ($P<0.05$). The efficiency of cardiovascular system to respond to noradrenaline was preserved and suitably amplified from 9 weeks to 52 weeks of age. The response to acetylcholine was amplified from 17 weeks to 52 weeks of age.

Key words: SHR, ontogenesis, noradrenaline, acetylcholine, vascular system

Introduction

Spontaneous hypertensive rats (SHR) are one of the most studied experimental models in cardiovascular pathology. Indeed, high blood pressure is generally accepted as one of the root risk factors of cardiovascular diseases. Discussion has been running on how far the experimental model of SHR, developed on genetical basis, does meet the human essential hypertension. Pros and contras have been formulated till present days. Differences discussed include body weight, occurrence of atherosclerosis, and before all, differences of prostaglandin mechanisms in renal circulation. All these issues require cautiousness in considering the identity of SHR and essential hypertension (Folkow et al. 1970, McGiff and Quilley 1981, Trippodo and Frohlich 1981, Labat et al. 2006).

Tenths of studies were devoted to reveal and identify, (i) the control mechanisms, and (ii) the tools, involved in maintaining the high blood pressure in SHR model. They comprise sympathetic nervous system (de Champlain et al. 1976, Folkow 1982, Scott and Pang 1983), renin-angiotensin system (Freslon and Giudicelli 1983, Wu and Berecek 1993), natriuretic peptides (Gutkowska et al. 1986), hormones (Willis and Bauer 1978), and recently relevant genes (Rapp 2000).

Several tenths of studies were devoted to geometry of resistant and conduit arteries, which the above mention disbalanced control mechanisms are operating on, and which are carrying the load of high blood pressure. The studies agree and confirm the old reckoning of Johnson, although in renal hypertension (1868), reaffirmed by Folkow (1970) that geometry of vessels, namely the increase in wall thickness and decrease of inner diameter in resistant arteries accompany the high blood pressure in SHR.

As far as structure of wall thickness increase concerns, both components: cellular and noncellular are suggested to participate (Lee et al. 1983, Kristek 1997). As far as smooth

muscle concerns, the experimental results varied from hypertrophy, hyperplasia, up to apoptosis (Lee et al. 1983, Dickhout and Lee 2000, Rizzoni et al. 2000).

Morphological composition of the respective vessel wall decides on its functional effectiveness – i.e. on the range of constriction and relaxation ability. Like the composition of vessels, the variation in vasomotor responses to stimuli of individual vessels in vitro, or perfused areas in vivo in SHR were demonstrated (Lais and Brody 1978, Tominaga et al. 1994, Yamazaki et al. 1999, Bund 2001, Gerová et al. 2005). The variability of results might reflect the fact that (i) animals used in experiments were in various phases of development (suckling, weaning, juveniles, adults), and (ii) various vascular portions were studied.

Blood pressure integrates the above structural and functional parameters in the whole vascular tree. This idea holds true in that case only if efficiency of the heart is stabilised. The heart weight increases with age in SHR more markedly than in Wistar rats. However, cardiac output is stabilised from 8 to 52 weeks as was demonstrated by Albrecht et al. (1972), Pfeffer at al. (1979).

The following experiments aimed to demonstrate the integrative blood pressure responses to basal constricting and relaxing stimuli in key developmental points in SHR and compare them with control Wistar rats.

Methods

Spontaneous hypertensive rats and Wistar rats as controls, males, were used for the experiments. Procedures and the experimental protocol were approved by the Animal Care Committee of the Slovak Academy of Sciences.

According to accepted critical ontogenetic periods in rats (Albrecht et al. 1972, Zicha and Kuneš 1999) following age groups were selected:

Group 1 - end of the suckling period - 3 weeks of age: the group consisted of 7 SHR and 8 Wistar rats.

Group 2 - end of the weaning period - 5 weeks of age: the group contained 7 SHR and 6 Wistar rats.

Group 3 - early adult age - juveniles - 9 weeks of age: group consisted of 7 SHR and 7 Wistar rats.

Group 4 - stabilised adult age - 17 weeks of age: group consisted of 7 SHR and 10 Wistar rats.

Group 5 - advanced adult age - 52 weeks of age: group consisted of 6 SHR and 5 Wistar rats.

For anaesthesia narkamon in the dose 0.25 ml/100g b.w., and rometar in the dose 0.1ml/100g b.w. were used, both administered i. p. Right jugular vein was prepared, cannulated, and ready to administer the respective drugs. Immediately, heparin in a dose of 25 i.u., was administered. Right carotid artery was prepared, an adequate cannula was introduced and connected to Stattham pressure transducer. Physioscript Schwarzer was used for recording the blood pressure.

After stabilization period lasting about 10 min, noradrenaline in the doses 0.1µg and 1.0µg, dissolved constantly in 0.1 ml of physiological salt solution, was administered into the jugular vein, over a constant period of 10 sec. Likely, acetylcholine in the doses 0.1µg, 1µg, and 10µg was administered in jugular vein. Both above drugs were administered in random order. Individual stimuli were applied in 10 - 15 minute interval, after BP returned to basic level and was completely stabilized.

Noradrenaline and acetylcholine were products of Sigma (Germany), heparin product of Zentiva (Czech Republic), narkamon and rometar for anaesthesia were produced by Spofa (Czech Republic).

The blood pressure values were expressed as means ± S.E.M. ANOVA and Bonferroni t-test was used for statistical evaluation. Values of $P<0.05$ were considered significant.

Results

Blood pressure in individual critical ontogenetic periods

The BP of SHR and Wistar rats in individual critical developmental periods demonstrates Fig 1. At the end of suckling period (3 weeks) the BP values in SHR reached 84.2± 6.3 mmHg and in Wistar rats 83.3±1.4 mmHg. The values are close each other. At the end of weaning period (5 weeks) the BP value found in SHR 105.0±3.4 mmHg, and 100.0±2.6 mmHg in Wistar rats, difference was not significant. The BP value 123.7±5.3 mmHg measured in juvenile SHR, 9 weeks of age, was significantly higher than blood pressure of Wistar rats 100.2±3.2 mmHg ($P<0.01$). A pronounced high blood pressure was found in adult SHR group 17 weeks of age, 176.6±9.6 mmHg, in age matched Wistar rats BP reached 107.6±3.6 mmHg only ($P<0.01$). The values found in 17 weeks old rats levelled in both SHR and Wistar rats. In the age of 52 weeks SHR presented 189.6±11.9 mmHg and Wistar rats 117.3±6.9 mmHg ($P<0.01$).

Blood pressure response to noradrenaline in individual critical ontogenetic periods.

Fig. 2 demonstrates the value of blood pressure increase after *0,1 µg noradrenaline* i.v. in individual age groups of SHR and Wistar rats. In the group of SHR 3 weeks of age after administration of noradrenaline the blood pressure increment value 8.3± 0.9 mmHg, and 8.8±1.8 mmHg in Wistar rats were very close. BP responses to the same noradrenaline dose in the group of 5 week old SHR 19.0±2.8 mmHg and 15.8±2.8 mmHg in Wistar rats indicate a tendency to differ. Blood pressure increase of SHR 9 weeks old was 22.5±2.7 mmHg, significantly higher in comparison to Wistar rats 11.2±2.3 mmHg ($P<0.01$). The response to noradrenaline in the group of 17 week old SHR represented 17.5±1.6 mmHg remarkably higher then 6.5±0.8 mmHg in Wistar rats ($P<0.01$), SHR 52 weeks of age responded to the

above noradrenaline dose by 19.7±2.0 mmHg and Wistar rats 7.4±1.9 mmHg (P>0.01), again the BP of SHR was significantly higher.

Fig. 3 represents the responses to *noradrenaline dose 1 μg i.v*. in SHR and Wistar rats of individual age groups. Noradrenaline elicited in SHR and Wistar rats 3 weeks old BP increase by 16.9±1.9 mmHg and 17.8±2.8 mmHg respectively. In 5 weeks old SHR and Wistar rats the response amounted 36.0±6.4 mmHg and 34.0±5.4 mmHg respectively, values close each other. SHR 9 weeks old responded to 1 μg noradrenaline by BP increase 67.8±9.5 mmHg and 42.6±6.25 mmHg (P<0.01). 17 weeks old SHR responded to 1 μg noradrenaline and Wistar rats by an BP increase 57.4±6.5 mmHg and Wistar rats by only 30.8±3.07 mmHg (P<0.01). Blood pressure increase elicited by 1.0 μg noradrenaline represented in SHR 52 weeks old 60.5±3.9 mmHg and in Wistar rats 40.5±3.2 mmHg (P<0.01), likely the quantitative difference was significant.

Blood pressure response to acetylcholine in individual critical ontogenetic periods.

Fig. 4 represents blood pressure decrease after *acetylcholine* i.v. in the dose *0.1 μg* in SHR and Wistar rats in individual age groups. 3 weeks old SHR responded to 0.1 μg acetylcholine by 29.4±3.3 mmHg and Wistar rats by 25.8±2.8 mmHg. 5 weeks old SHR responded by 35.1±4.3 mmHg and Wistar rats 25.0±1.4 mmHg. SHR 9 weeks old responded by 34.6±3.5 mmHg decrease and Wistar rats by 39.8±3.8 mmHg. However, response of 17 weeks old SHR represented 76.2±4.0 mmHg and Wistar rats only 48.6±2.5 mmHg (P<0.01) quantitatively marked difference. Similar relation were found in SHR 52 weeks old, acetylcholine induced hypotension by 69.9±10.2 mmHg, and Wistar rats only 37.4±4.2 mmHg (P<0.01).

Fig. 5 represents responses to *acetylcholine 1 μg* i.v. in SHR and Wistar rats in individual age groups. 3 weeks old SHR responded by 42.7±3.8 mmHg and Wistar rats 37.4±3.0 mmHg. 5 weeks old SHR and Wistar rats responded by 62.0±5.7 mmHg and 51.3±9.2 mmHg

respectively. 9 weeks old SHR and Wistar rats responded by 59.1±3.1 mmHg and 53.1±2.4 mmHg respectively. However 17 weeks old SHR responded by 96.6±10.1 mmHg and Wistar rats only 60.4±2.9 mmHg ($P<0.01$). SHR 52 weeks old responded by 87.5±11.8 mmHg and Wistar rats by 62.3±3.5 mmHg ($P<0.01$).

Fig. 6 represents responses to *acetylcholine10 µg* i.v. of SHR and Wistar rats in individual groups. 3 weeks old SHR and Wistar rats responded by 51.6±2.9 mmHg and 44.5±2.3 mmHg respectively. 5 weeks old SHR and Wistar rats responded by 74.0±7.2 mmHg and 66.5±6.9 mmHg respectively. 9 weeks of age yielded hypotension 65.6±6.1 mmHg and Wistar rats 62.9±3.9 mmHg still close each other. 17 weeks old SHR responded by 126.5±10.3 mmHg BP decrease and Wistar rats only 68.5±2.7 mmHg ($P<0.01$). 52 weeks old SHR responded by 103.4±10.6 mmHg and Wistar rats to the same dose by 73.5±2.8 mmHg ($P<0.05$) BP decrease.

Discussion

The blood pressure value of 3 weeks and 5 weeks old SHR, till the end of weaning period, did not differ from that found in the age matched Wistar rat offspring. However, a significant difference in the blood pressure value was found between 9 weeks old SHR and Wistar rats. High BP in SHR was maintained till 52 week.

Blood pressure response of sucklings 3 weeks old to noradrenaline administered in two increasing doses (0.1µg and 1µg), increased dose dependently in the same range in SHR and control Wistar rats. Neither difference in BP response to noradrenaline was found even in 5 weeks old weaning SHR and Wistar rats.

However, the juvenile SHR, 9 weeks old, responded to both doses by an amplified increment of blood pressure in comparison to Wistar rats. As was mentioned above, this age

group of SHR is characterized already by a significantly higher value of steady state blood pressure.

The amplified range of BP response to noradrenaline expressed in mmHg persisted in adult 17 weeks old SHR, and even the 52 weeks old SHR yielded a similar amplified pattern of response to noradrenaline. Calculation the responses in relation to steady state pressure yielded similarly amplified value in SHR.

Hypotension responses to increasing doses of acetylcholine (0.1µg, 1µm, and 10µg) increased dose dependently in 3 weeks old SHR and Wistar rats. No difference was found between the responses of the two strains studied. Neither difference in hypotensive response extent to individual doses of acetylcholine in SHR and Wistar rats was found in 5 weeks old offspring, or even in 9 weeks old animals. In spite a clear-cut, significantly higher steady state blood pressure was found already in 9 weeks old SHR, in comparison to Wistar rats. And more over, the 9 weeks old SHR yielded a significantly amplified response to noradrenaline in both doses, in comparison to control Wistar rats.

The significantly amplified hypotension to all three doses of acetylcholine was found only in 17 weeks old SHR, and also in 52 week old SHR, when compared to Wistar rats.

There is an almost generally accepted view that in SHR blood pressure starts to increase after 5 weeks notably and achieves a significant different value in comparison to Wistar rats (Zicha and Kuneš 1999 see for review). There is also a common agreement as far as concerns the biophysical background of high blood pressure. The geometry of resistant vessels, namely increased wall/lumen ratio was confirmed in several studies (Folkow et al. 1970, Mulvany and Aalkjaer 1990, Bund 2001). However, there is not a unified view as far as concerns the component responsible for wall thickness increase. Indeed, the respective components surely should affect vasomotor efficiency of vascular system. Dickhout and Lee (2000) found even in 4 weeks old offspring of spontaneous hypertensive parents difference in length of smooth

muscle cells in mesenteric vessels. Lee et al. (1983) demonstrated in several studies that in mesenteric area, in particular in muscular arteries and arterioles, hypertrophy, and also hyperplasia of smooth muscle cells was found in 12 weeks old animal.

Brilla et al. (1991) and Weber (2000) confirmed the hypertrophy and hyperplasia of smooth muscle cells in media of intramural coronary arteries in cardiac hypertrophy of adult SHR. They drew attention on the contribution of non-cellular component, to the wall thickness increase. Recent analytical studies, using the point counting method, yield further reliable data on increase of non-cellular, and/or extracellular matrix in conduit vessel wall of adult SHR, and also in other experimental hypertensive models (Kristek 1997, Kristek et al. 2006). Thus, the data indicating the contribution of extracellular matrix, beside the media smooth muscle, to vascular wall thickness have to be faced up.

As a source of extracellular matrix fibroblasts might be hypothetically considered. One supporting point emerges: (i) Sympathetic nervous system has been considered as one of the ground mechanisms responsible for (underlying) the high blood pressure in SHR (de Champlain et al. 1976, Scott and Pang 1983, Lee et al. 1986, Dominiak et al. 1987, Head 1989). (ii) Fibroblasts in vascular wall have much close apposition to adrenergic nerve terminals, contrary to vascular smooth muscle (Kristek and Gerová 1987). (iii) Fibroblasts are endowed with adrenergic receptors (Azevedo and Soares-da-Silva 1981). However, the effect of sympathetic nervous system on function of fibroblasts is not known, and remains open as hypothesis.

The other source of extracellular matrix increase in vascular wall might be vascular smooth muscle cell itself. That one due to transformation of phenotype from contractile to synthetic type might also participate in production of extracellular matrix and thereby wall thickness increase. The question of contractility alterations due to change of phenotype of vascular smooth muscle is also a challenging issue.

As far as the contractile efficiency concerns, one of the first data by Lais and Brody (1978) provided evidence on hyperresponsiveness to noradrenaline in hindquarter resistant area of 3 weeks old spontaneous hypertensive rats. Similar results offer the study by Dickhout and Lee (2000) on mesenteric vessels of 3-4 weeks old offspring of hypertensive parents and compared to control Wistar age matched rats. The results of experiments on mesenteric arteries were not confirmed by Bund (2000). And further, Bund (2000) found no difference in basal myogenic tone of femoral artery from spontaneous hypertensive rats and control Wistar rats of 4-5 month of age. Tahvanainen et al. (2006) provided data dealing with response of isolated human mesenteric arteries. He found in vitro an increased response to noradrenaline, KCl, and serotonin.

Our experiments demonstrated that during the development from 3 weeks up to 52 weeks SHR maintain the vascular constriction ability. The BP response to noradrenaline in SHR from 3 weeks to 5 weeks of age was in a comparable range with Wistar rats. From 9 weeks to 52 weeks, however, SHR responded by a mild significantly amplified increase of BP.

As far as Ach relaxation concerns Pourageaud and Freslon (1992) found in both mesenteric arteries and coronary arteries of SHR a reduced relaxation to Ach. They stated that the cause was functional alteration of the respective endothelium, whereas the efficiency of smooth muscle cells did not differ from Wistar rats. Endothelium dependent relaxation was found to be reduced in conduit vessels: aorta of SHR by Lüscher and Vanhoutte (1986) and in cerebral arteries by Tesfamariam and Halpern (1988). Also Qiu et al. (1998) found an attenuation of flow induced dilation, generally accepted as NO underlying phenomenon.

Contrary to the above studies, carried out mostly on isolated arteries, results of experimental studies in vivo on anaesthetized SHR demonstrate an enhanced hypotension to acetylcholine expressed in mmHg, in comparison to control Wistar rats. Granstam et al. (1998) in an extensive study of haemodynamic of SHR demonstrated a significantly enhanced

hypotensive response of systemic BP to acetylcholine in 12 weeks old spontaneously hypertensive rats. Granstam et al. (1998) work confirmed the experiments of Yamazaki et al. (1991) providing data on mildly amplified hypotension induced by acetylcholine in SHR 6 and 13 weeks old. Controversial are experiments of Tominaga et al. (1994), who described decreased BP response in 4 month and 8 month old SHR after acetylcholine.

Our results from five series of SHR descendants aged 3, 5, 9, 17 weeks up to 52 weeks proved sufficient efficiency of vasodilation mechanism as reflected by BP decrease to three increasing doses of acetylcholine, from the first weeks of life. In 17 weeks and 52 weeks old SHR the response was amplified. Moreover, in an other study the SHR (10 weeks old) treated for six weeks with NO-donors, yielded also an enhanced hypotension after acetylcholine compared to Wistar control rats (Gerová et al. 2005). Mildly amplified Ach response was revealed not only in rats, but also in aortas of dog puppies 6 weeks old (Török and Gerová 1996).

It is noteworthy that (i) significant steady state BP increase, and (ii) significantly amplified constrictor response to both doses of noradrenaline, was found in 9 weeks old SHR. However significant increase of hypotension to all three doses of acetylcholine was found only in 17 weeks old SHR. The above time sequence of vasomotor activities reflected by BP raises the consideration whether the increased vasoconstrictor activity, underlied by proven sympathetic nervous system and RAS, does activate NO synthase, does trigger an increase of arginine metabolism in endothelial cells, with consequent increase of NO production – as a process of adaptation.

It is for sure, reckoning the blood pressure integrative response in SHR and Wistar rats: either vasoconstriction to noradrenaline or vasorelaxation to acetylcholine, these responses do not reveal signs of failure of the cardiovascular system from 3 up to 52 weeks of rat age. Contrary, the efficacy of vascular system to respond to vasomotor stimuli seems to be ready

and even higher suggesting the adaptation process. Nevertheless, this surprising findings maintain the challenge for the intensive studies of clinical physiology and pathophysiology of human hypertension.

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Legend to Figures

Fig.1 Steady state BP in individual critical ontogenetic periods.

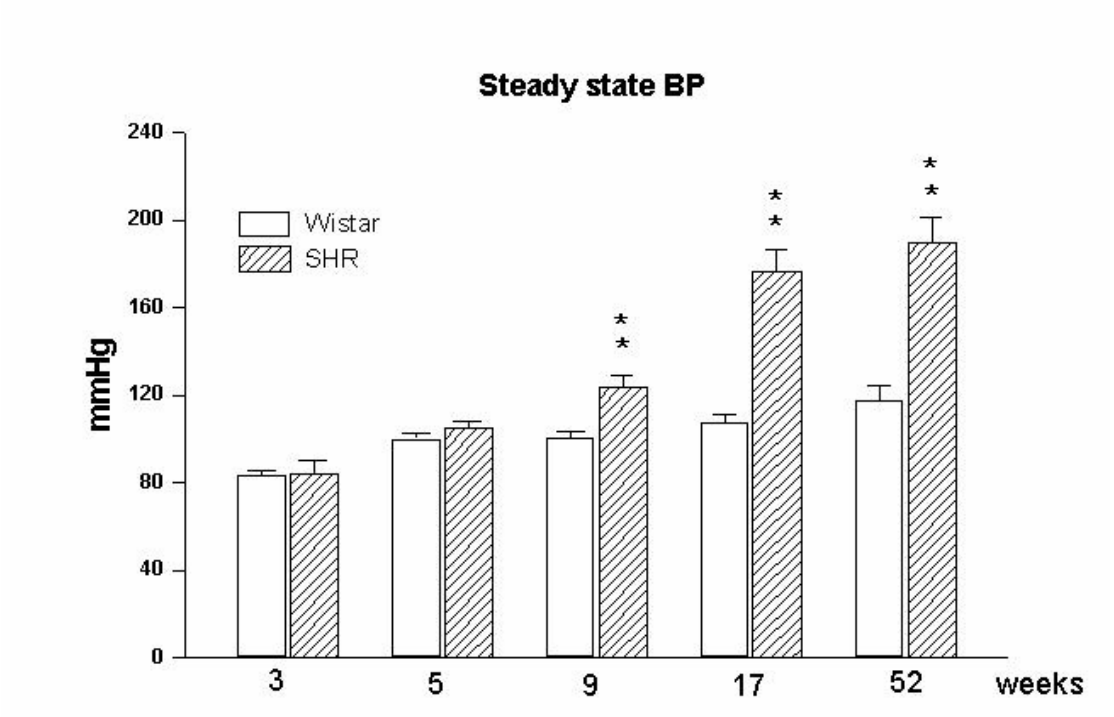
Fig.2 BP increase induced by noradrenaline 0.1µg i.v. in individual critical ontogenetic periods.

Fig.3 BP increase induced by noradrenaline 1µg i.v. in individual critical ontogenetic periods.

Fig.4 BP decrease induced by acetylcholine 0.1µg i.v. in individual critical ontogenetic periods.

Fig.5 BP decrease induced by acetylcholine 1µg i.v. in individual critical ontogenetic periods.

Fig.6 BP decrease induced by acetylcholine 10µg i.v. in individual critical ontogenetic periods.


Steady state BP
240
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mmHg
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3
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9
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weeks

Fig. 1

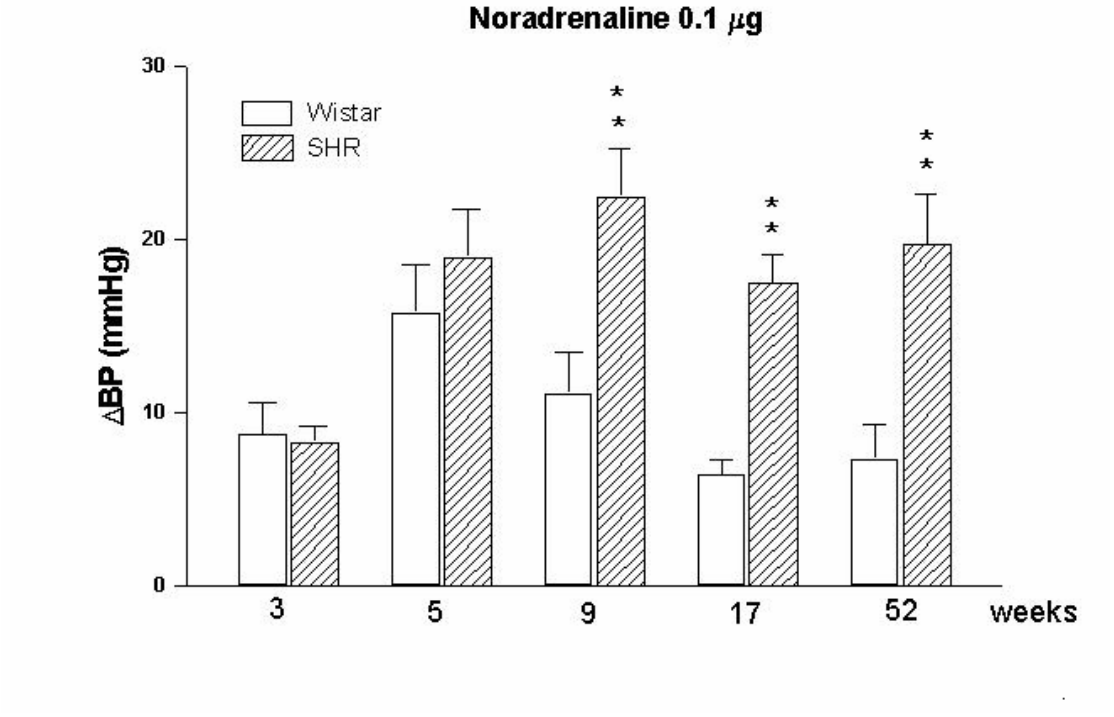
Noradrenaline 0.1 μg
30
20
10
0
ΔBP (mmHg)
Wistar
SHR
* *
* *
* *
3
5
9
17
52
weeks

Fig. 2

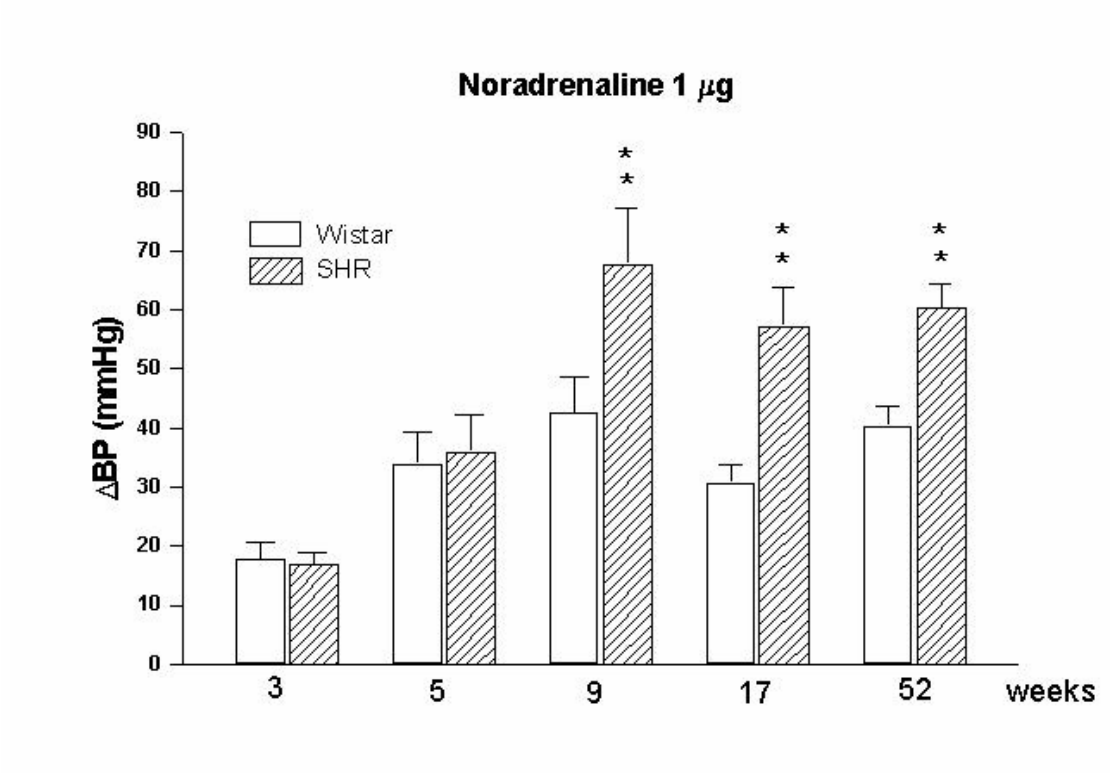
Noradrenaline 1 μg
ΔBP (mmHg)
90
80
70
60
50
40
30
20
10
0
Wistar
SHR
* *
* *
* *
3
5
9
17
52
weeks

Fig. 3

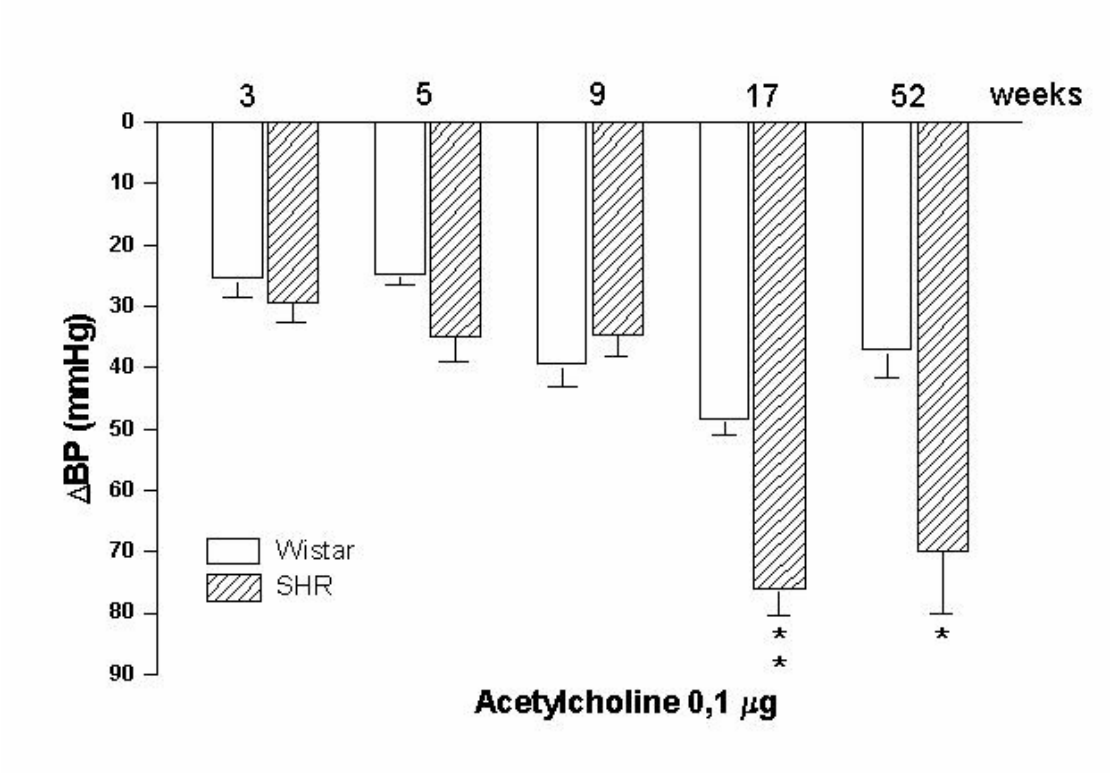
3
5
9
17
52
weeks
0
10
20
30
40
50
60
70
80
90
ΔBP (mmHg)
Wistar
SHR
* *
*
Acetylcholine 0,1 μg

Fig. 4

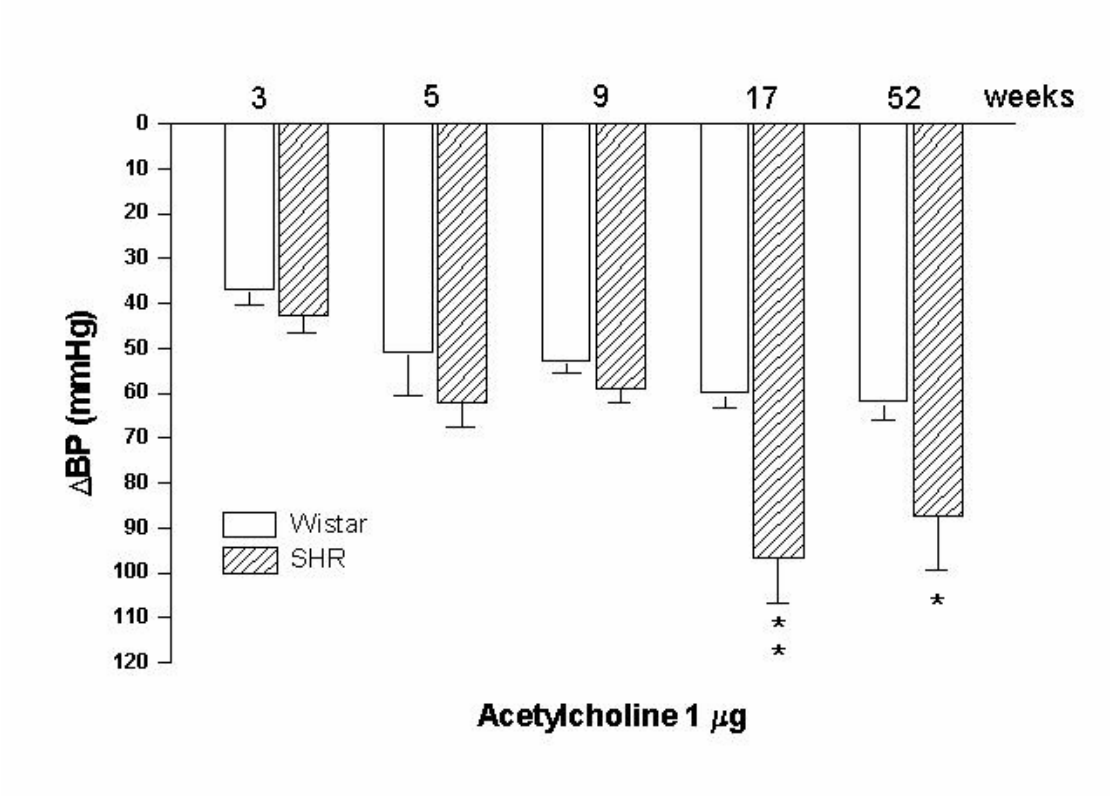
3
5
9
17
52
weeks
0
10
20
30
40
50
60
70
80
90
100
110
120
ΔBP (mmHg)
Wistar
SHR
*
*
*
Acetylcholine 1 μg

Fig. 5

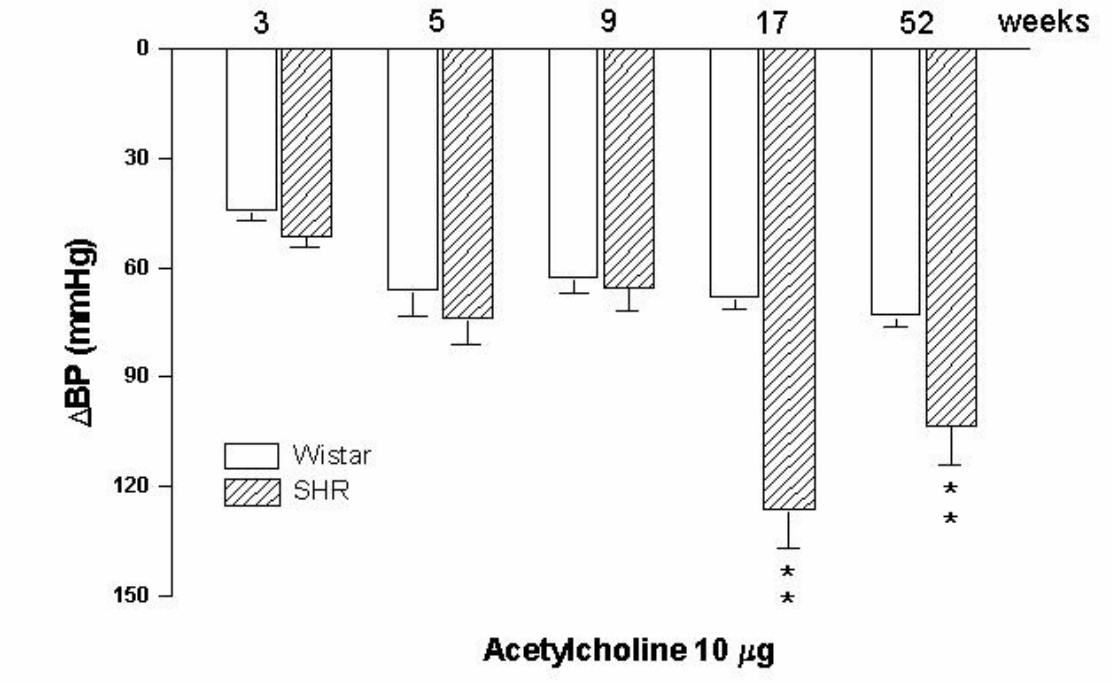
3
5
9
17
52
weeks
0
30
60
90
120
150
ΔBP (mmHg)
Wistar
SHR
*
*
*
*
Acetylcholine 10 μg

Fig. 6